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Previews

Chromatin Modifiers, Cognitive Disorders, and Imprinted Genes

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In this issue of *Developmental Cell*, Kernohan et al. link the chromatin regulatory proteins ATRX, MeCP2, CTCF, and cohesin with silencing of H19 and other imprinted genes during critical stages of postnatal brain development, perhaps suggesting a common etiology for several human diseases that exhibit defects in brain development and function.

Chromatin architecture and genome organization play essential roles in establishing the gene expression patterns that are critical for normal development and function. A number of human disorders have been identified whose primary genetic defect is in loci whose gene products regulate chromatin and chromosome architecture. Examples include Rett syndrome, which is associated with loss of function of the methylated-DNA binding protein MeCP2; Cornelia de Lange syndrome, which is associated with mutations in the cohesin-related factors SMC1A, SMC3, and NIPBL; and Alpha-Thalassemia mental Retardation, X-linked (ATR-X), which is associated with mutations in ATRX, an ATP-dependent chromatin remodeling protein. All three of these diseases present broad and partially overlapping phenotypes that include deficits in brain development and function. While the primary regulatory defect for each of these syndromes has been determined, the challenge still exists to identify the downstream targets of these genes with the goal of eventually developing appropriate therapies. In this issue of *Developmental Cell*, Kernohan et al. (2010) analyze the roles of ATRX, cohesin, and MeCP2 at the imprinted *H19* gene and demonstrate that these factors interact together with the transcription factor/insulator protein CTCF to regulate expression of an entire network of imprinted genes. This leads to the intriguing hypothesis that changes in expression of imprinted genes may contribute to the brain defects associated with these diseases.

The *Igf2*/*H19* locus is a typical imprinted gene cluster (Wan and Bartolomei, 2008). It includes paternally expressed genes, *Insulin2* and *Insulin-like growth factor 2* (*Igf2*), and one maternally expressed gene, *H19*. The maternal and paternal chromosomes differ not only in gene activity but also in chromosome architecture. They display distinct patterns of DNA methylation, histone modification, and DNA loop formation. All of the differences across the >100 kb locus depend entirely upon a 2.4 kb element, the *H19* Imprinting Control Region (*ICR*) that lies just adjacent to the *H19* promoter and about 100 kb upstream of *Igf2* and *Insulin2*. Deletion of the *ICR* renders the two chromosomes indistinguishable, while insertion of this element ectopically will result in artificial imprinting of the targeted locus. Maternally inherited *ICR* DNA is not methylated and binds the CTCF transcription factor in a cohesin-dependent manner, resulting in expression of *H19* in the embryo and early neonatal stages. Moreover, CTCF-bound maternal *ICR* interacts through DNA looping with the *Igf2* promoter region, preventing *Igf2* promoters from physically interacting/looping with downstream enhancers. In contrast, paternal *ICR* DNA is methylated and therefore cannot bind CTCF. Since CTCF-dependent loops are not formed between the *ICR* and the *Igf2* gene on the paternal chromosome, the *Igf2* promoters are instead free to interact with the downstream enhancers and activate paternal expression. In addition, the methylated *ICR* induces chromatin changes at the adjacent *H19* promoter that prevent its activation.

Besides these intrachromosomal interactions, recent studies have demonstrated CTCF-dependent interchromosomal interactions between the maternal *H19 ICR* and other loci, with a particular enrichment for imprinted genes (Ling et al., 2006; Sandhu et al., 2009; Zhao et al., 2006). Thus there is quite a lot going on at the *H19 ICR*, and Kernohan et al. demonstrate that the element also represents a locus of integration, linking diverse disease loci.

Based on initial findings that *H19* and *Igf2* were significantly upregulated in *ATRX*$^{-/-}$ mice, Kernohan et al. characterize protein complexes at the *H19 ICR*. They demonstrate that in addition to cohesin and CTCF, ATRX and MeCP2 also bind specifically to the maternal (i.e., non-methylated) *ICR*. Furthermore, cohesin and CTCF binding are ATRX dependent. The MeCP2 binding to the maternal ICR is surprising, since it was always assumed that this CpG methyl-binding protein interacted specifically with the methylated paternal *ICR*. Kernohan's results thus support newer hypotheses that MeCP2's primary role is in forming DNA loops that stimulate gene activity and is not limited to mediating DNA-methylation-induced repression (LaSalle, 2007).

The increase in *H19* expression in ATRX-deficient mice is maternal in origin, i.e., exhibits no loss of imprinting. This implicates ATRX in postnatal repression of *H19* (and possibly *Igf2*). After birth, expression of these two genes is repressed hundreds of fold. The biological necessity for this repression is absolute, but the underlying mechanisms have been elusive until now. The authors do not determine the parental origin of the extra *Igf2*, but their results predict that it is likely maternal in origin.

Kernohan et al. also demonstrate interactions of cohesin, MeCP2, and ATRX

proteins at the *Glt2 ICR*, although in this case their analyses did not allow them to determine whether these interactions occur on the maternal or on the paternal chromosome. The *Dlk1/Glt2* locus is somewhat analogous to the *Igf2/H19* locus in gene organization and regulation (Wan and Bartolomei, 2008). An *ICR* adjacent to the *Gtl2* promoter regulates *Gtl2* and the far upstream *Dlk1*. The results of Kernohan et al. support the idea that interactions of cohesin, MeCP2, and ATRX proteins are of general importance and not restricted to *H19/Igf2*. However, several differences between the two loci suggest that the nature of these interactions may not be straightforward. At *Dlk1/Glt2*, the proteins each bind to distinct parts of the *ICR* and not to a single region, like they do at the *H19* locus. Furthermore, MeCP2 binding to the *ICR* is ATRX dependent at *Glt2*, but ATRX independent at *H19*.

By several criteria, *Igf2*, *H19*, *Dlk1*, and *Glt2* are part of a network of at least 10 imprinted genes (Varrault et al., 2006). These genes all share developmental and tissue-specific patterns of expression and respond similarly to mutations at the *Zac1* locus. Curiously this network shows almost no overlap with imprinted genes involved in interchromosomal interactions with the *H19 ICR*. Kernohan et al. provide good evidence that ATRX is required for the downregulation of expression of each of these genes in late embryonic or in postnatal development. The key question remains whether this downregulation is important in the ATR-X syndrome.

Evolutionary theory and the analysis of many knockout mouse strains both support the idea that the primary effect of imprinting is on fetal and early neonatal growth. However, some experiments have suggested a role for imprinted genes in brain development and function (Wilkinson et al., 2007). Chimeric animals generated by mixtures of wild-type and gynogenetic (maternal chromosomes only) cells, or by mixtures of wild-type and androgenetic (paternal chromosomes only) cells, show divergent phenotypes with gynogenetic and androgenetic cells each contributing to distinct brain structures (Keverne et al., 1996). These experiments are hard to interpret on a molecular level. Gynogenetic cells not only lack any paternal-specific transcripts, but also have 2-fold overexpression of all maternal-specific RNAs. Nonetheless the results are intriguing. Mammalian cells go to great effort to carefully regulate the doses of imprinted genes. Whether misexpression of *H19* or of any of the imprinted genes plays a clinically important role in brain development and function is an important and difficult question that remains to be addressed.

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A PAK-Activated Linker for EGFR and FAK

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Transmembrane growth factor and integrin matrix receptors form multiprotein signaling complexes with FAK, a cytoplasmic cell motility-associated kinase. In a recent issue of *Molecular Cell*, Long et al. now show that a PAK-phosphorylated alternate-spliced isoform of the steroid receptor coactivator-3 (SRC-3Δ4) bridges EGFR and FAK, enhancing breast carcinoma cell migration and metastasis.

Cell movement results from the coordination of actin cytoskeletal and cell adhesion site formation-turnover alterations generating shape and traction force changes. Focal adhesion kinase (FAK) is a cytoplasmic tyrosine kinase that colocalizes with and is activated by integrin matrix receptors at adhesion sites. For a cell to process motility-promoting stimuli correctly, there must be essential proteins that function as "integrators" in the coordination of signals regulating cell shape, adhesion, and cell motility. FAK is one such integrator linking transmembrane integrin, growth factor, and G protein-linked receptors to the cell motility machinery (Mitra et al., 2005). FAK is required for efficient epidermal growth factor (EGF)-stimulated cell motility and this connection is facilitated through FAK FERM (band 4.1, ezrin, radixin, moesin